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(57) Abstract

An antibiotic sensitivity profile for pathogens is obtainable by a test comprising the steps of capturing bacteria, introducing a selected antibiotic in a predetermined inhibitory amount to the captured bacteria in a suitable cultivation medium, incubating the bacteria under conditions conducive to normal growth of the bacteria, and testing for the presence of ATP to determine the viability of the bacteria culture.

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ANTIBIOTIC SENSITIVITY PROFILE

This invention relates to an antibiotic sensitivity profile and in particular to a rapid antibiotic sensitivity profile for pathogens.

Following isolation and identification of a pathogen, it is often necessary to carry out *in vitro* tests to determine the susceptibility of the pathogen to each of a range of appropriate antibiotics. The results of such tests assist the clinician in the selection of optimally active agents for administration. The most common method of sensitivity test is to use agar plates inoculated with the particular pathogen under test.

Initially a swab or other sample containing the pathogen is inoculated onto a series of agar plates. The plates are then incubated either aerobically or anaerobically for 48 hours. Assuming a selective medium has been used, any cultures should be the presumptive pathogen. When sufficient growth is available one further culture is made to provide a purity plate for antisera testing and a second is inoculated onto sensitivity test agar; this is a solid medium which contains no constituents which excessively enhance or reduce the inhibitory or lethal effects of the particular antibiotics tested. Onto such a sensitivity agar plate are placed several small absorbent paper discs, each impregnated with a different antibiotic. The plates are then incubated for a further 48 hours. During subsequent incubation the antibiotics diffuse out into the surrounding agar, and zones of growth inhibition occur around those discs which contain antibiotics to which the organism is sensitive. The diameter of these zones is then measured to calculate the degree of sensitivity or resistance to that particular antibiotic.

In an alternative form of the test, solutions of antibiotics are placed, individually, into a number of wells cut into the agar.

The disadvantages of these prior art tests are that they are slow and cumbersome. A further disadvantage arises where the pathogen under test is a strain of *Staphylococcus aureus* which happens to be penicillin resistant and therefore commonly forms zones of growth-inhibition around discs containing penicillin. In such a case these no growth zones are produced because penicillinase-production in such strains is inducible; thus, cells close to the penicillin disc, which are exposed immediately to high concentrations of the antibiotic are killed before adequate amounts of penicillinase can be synthesized. This activity can thus produce a false-positive result.

The aim of this invention is to provide an antibiotic sensitivity test which obviates or mitigates these disadvantages.

This object is achievable according to the invention by a test comprising the steps of capturing bacteria, introducing a selected antibiotic in a predetermined inhibitory amount to the captured bacteria in a suitable cultivation medium, incubating the bacteria under conditions conducive to normal growth of the bacteria, and testing for the presence of ATP to determine the viability of the bacteria culture.

The method of bacteria capture is important but not critical since a number of alternatives are available, including use of an affinity column, or a microfiltration membrane which is optionally blocked in a manner known *per se* (so-called "dead end filtration"), or an in-line air filter, or a cross-flow and flow-through filtration technique, again optionally using blocking buffer reagents. Suitable support media such as beads, particles, fibres,

films and membranes may be formed from cellulose-acetate, cellulose-nitrate, regenerated cellulose, polysulphone, polyacrylonitrile, polyamide, polyimide or the like.

Thus according to one aspect of the invention there is provided a method of determining the antibiotic sensitivity of bacteria comprising, contacting a sample to be tested with selected supported mono-clonal antibodies for a sufficient period of time to permit binding, recovering the sample containing bound target material, mixing the said sample with nutrient broth containing an antibiotic, incubating the mixed sample and subsequently testing the sample for the presence of ATP.

Testing is suitably carried out by use of optical detection means, such as a luminometer or spectrometer, the sample being treated with a bioluminescent or chemiluminescent reagent system. The optical detection system would be one tunable to detect the wavelength of emission appropriate for the luminescent reagent system.

The invention will now be described hereinbelow by way of illustration with reference to one specific embodiment.

An affinity column is provided which contains purified highly specific mono-clonal antibodies fixed to glass or other such support beads. The mono-clonal antibodies provided are specific for one particular pathogen i.e. *Staphylococcus aureus* to allow any of the said bacteria in a sample to be retained in the affinity column.

Test fluids, obtained by, for example, rinsing swabs in liquid nutrients, or obtaining body fluids directly for testing, are passed through the column an amount in the range of 1 ml - 1000 ml for up to 1 hour.

The captured bacteria are eluted from the column using 1 ml of a suitable liquid of a different pH from the column.

This step takes approximately 2 minutes during which time the pH is brought to near neutral.

Two or more micro tubes of nutrient broth each with 100 µl of the eluted bacterial suspension are added together with known LD100, (Lethal Dose 100%) amounts of antibiotics. The tubes are then incubated at the optimum temperature of the particular bacteria for 8 hours. This incubation is carried out aerobically or anaerobically depending on the growth conditions favoured by the test bacteria.

Following incubation a rapid test is carried out to detect the presence of Adenosine Triphosphate (ATP), in this case using a bioluminescent technique employing luciferase luciferin L7. In this test 100 µl of the incubated nutrient both containing the concentrated bacteria and the antibiotic are inoculated with 0.02% benzalkonium chloride in order to disrupt the bacterial membranes. A 10 mg/ml aqueous solution of firefly lantern extract is prepared and a 100 µl amount is dispensed into a suitable cuvette. This cuvette is placed in a luminometer in which the wavelength has been set at 560 nm, and thus any background luminescence can be determined and the luminometer zeroed accordingly. An amount (20 µl) of the bacterial sample is added to the cuvette and mixed therein. After 20 seconds the light emission is measured. This test is carried out on each antibiotic to be tested and takes approximately 3 minutes to complete. If no light is emitted from the cuvette this indicates that no ATP is present and therefore no live bacteria are in the sample. If bacteria are present in the sample then light will be emitted and a reading taken from the spectrometer. The degree of sensitivity of the bacteria to various antibiotics can then be determined by using a standard curve of light emission against the bacterial concentration.

The advantages of this method are that the time for the new method is approximately 9 hours whereas the prior art

method takes at least 87 hours to allow for the growth of the cultures. Also through use of the mono-clonal antibodies the operator is assured that only specific organisms are isolated thereby negating any need for purity or serological testing.

A test kit for performing the rapid antibiotic sensitivity test may include,

(i) support means, such as a membrane or fibre filter, prepared for capture of bacteria and containment means therefor;

(ii) a range of antibiotics contained in a plurality of containers, or a plurality of compartments in a container, each antibiotic being provided in an amount predetermined to be inhibitory for a selected susceptible bacterium;

(iii) culture medium suitable for cultivation of the target bacteria; and

(iv) a bioluminescent or chemiluminescent reagent for determining the presence of ATP optically to thereby determine the viability of the bacteria culture.

Claims

1. A rapid antibiotic sensitivity test comprising the steps of capturing bacteria, introducing a selected antibiotic in a predetermined inhibitory amount to the captured bacteria in a suitable cultivation medium, incubating the bacteria under conditions conducive to normal growth of the bacteria, and testing for the presence of ATP to determine the viability of the bacteria culture.

2. A test according to claim 1 wherein the step of capturing bacteria comprises the use of an affinity column wherein purified target bacteria specific mono-clonal antibodies are fixed to support means.

3. A test according to claim 1 wherein the step of capturing bacteria comprises the use of a membrane in a dead-end filtration technique.

4. A test according to claim 1 wherein the step of capturing bacteria comprises the use of an in-line air filter, or a cross-flow and flow-through filtration technique.

5. A test according to claim 3 or claim 4 wherein the bacteria are captured on a medium pre-treated with a blocking agent.

6. A method of determining the antibiotic sensitivity of bacteria comprising, contacting a sample to be tested with selected supported mono-clonal antibodies for a sufficient period of time to permit binding, recovering the sample containing bound target material, mixing the said sample with nutrient broth containing an antibiotic, incubating the mixed sample and subsequently testing the sample for the presence of ATP.

7. A method according to claim 6 wherein the presence of ATP is detected optically, the sample being treated for this purpose with a bioluminescent or chemiluminescent reagent system.

8. A method according to claim 7 wherein the bioluminescent reagent is a luciferase/luciferin e.g. L7.

9. A test kit for performing a rapid antibiotic sensitivity test including,

(i) support means prepared for capture of bacteria and containment means therefor;

(ii) a range of antibiotics contained in a plurality of containers, each antibiotic being provided in an amount predetermined to be inhibitory for a selected susceptible bacterium;

(iii) culture medium suitable for cultivation of the target bacteria;

(iv) a bioluminescent or chemiluminescent reagent for determining the presence of ATP optically to thereby determine the viability of the bacteria culture.

10. A kit according to claim 9 wherein the support means prepared for capture of bacteria includes a membrane or fibre filter.

11. A kit according to claim 9 including a blocking buffer in a suitable container.